REMARKS

Applicant has carefully reviewed and considered the Office Action mailed on October 20, 2005, and the references cited therewith.

Claims are amended, and claims 88, 89, 91, 92 and 96-123 are canceled; as a result, claims 87, 90, and 93-95 are now pending in this application.

35 U.S.C.112, 1st Paragraph, Rejection of the Claims

Claims 87, 89, 90, and 96-98 were rejected under 35 USC ¶112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. However, the Examiner provided some claim language which would be patentable thereunder. Applicants have somewhat broadened this claim (#87) with language from page 46, first full paragraph. The whereby clause is amply supported by Tables I and II of gene expression associated with neurogenesis and blood cells, respectively.

Applicants are uncertain as to the meaning of the comment in the Office Action on page 3, lines 7-8, that: "For clarity, it is noted that culturing the cord blood mononuclear cells with retinoic acid and NGF does not fall within the scope of claim 87." By amendment, both compounds are mentioned in the claim. Further, the experiment on page 46 (lines 13-15) describes the use of not only retinoic acid and NGF, but also BDNF and GDNF. Hence, all these compounds are appropriately recited in the claim.

The Office Action alleges that the specification fails to provide an enabling disclosure for the methods of making neural cell compositions because the only use is therapeutic transplantation and goes on to question the efficacy of such techniques and adequacy of guidance. The neural compositions are used in the well respected animal model rate MCAO model (or TBI), whose Rotarod test and NSS "are generally used for the evaluation of the effects of the drugs on the behavioral responses after TBI and stroke in animals." (page 72, lines 2-8) In the Results section, the effect of the administration of treated umbilical cord cells was compared to treatment with traumatic brain injury alone at 14 and 28 days after TBI and HUBC treatment. Rotarod scores were significantly improved with NGF+RA HUBC treatment ($p<0.05$). Likewise, the neurological severity scores were also significantly improved with NGF+RA

HUBCs ($p<0.05$). Because the transplantation of the cells made by the inventive method actually had a salutary effect on brain function, prior authors with reasons and rationales for the treatment to lack function should not be given much weight. Nevertheless, the Applicants will briefly discuss the cited references.

The Office Action cites Jackowski (1995) for limitations and unpredictability of neural tissue transplantation. Aside from the fact that huge progress has been made in the period between the drafting of that article and the filing of the instant patent application, Jackowski notes the "ability of certain mammalian central neurons to regenerate successfully...certain adrenergic, noradrenergic, dopaminergic and serotonergic neurons seem to be capable of sustaining a more prolonged regenerative growth response." (page 307, 1st paragraph). More specifically, Jackowski notes that "donor embryonic hippocampal, or septal neurons from more than one species and also *human neuroblasts*, when transplanted into an adult mammalian CNS environment can form axons which appear able to grow into and innervate either local or distal target fields within a host animal." (emphasis added; page 308, 2d full paragraph). Likewise, he states that "[s]ympathetic and most, if not all, neural crest-derived sensory neurons require NFG for survival during embryonic and early postnatal life... NGF receptors are also widely present and play a significant role in mammalian CNS development, particularly in the case of cholinergic neurons." (page 308, column 2) The instant invention calls for treating the neuron-like cells with NGF which contributes to their survival. Nevertheless, in spite of the drawbacks highlighted by the Office Action, Jackowski admits that *"CNS implants in adult brains survive"*, although they "function less well than in newborn animals." (emphasis added; page 309, column 2) In conclusion, Applicants believe that Jackowski's comments support implanting early-stage neuron-like cells treated with NGF, such as the cells prepared by the claimed method.

Grados-Munro et al. is cited for the proposition that axon outgrowth inhibition is a major barrier to axon regeneration in the CNS. As the Office Action states, the authors contemplate that a combination of approaches, including treatment to neutralize the inhibitory character of the CNS environment, may be required for CNS regenerative therapy (page 479). Since the inventive cells are derived from young umbilical cells, it is pertinent that Grados-Munro mentions that some mice (in particular, young mice) show substantial corticospinal sprouting proximal to a spinal cord dorsal hemisection in a number of corticospinal axons below the lesion.

(page 481, middle of column 1). Hence, given this report and the attributes of neuron-like cells prepared by the inventive method, their salutary effect on transplantation is not surprising.

Filbin is cited as disclosing inhibitors of axonal regeneration. However, in the abstract, Filbin notes that "New molecular information has also accumulated on how the neuron can be changed intrinsically to overcome myelin inhibitors." (page 1) Filbin describes the situation with young neurons (similar to the neuron-like cells produce by the inventive method), "at an age when they are not inhibited by MAG or myelin in general and can spontaneously regenerate cAMP levels are high....and neurite outgrowth is actually promoted." (page 9, column 2) This latter comment seems most pertinent to these inventive young HUCB cells and may even support the evidence in the application that cells produced by the inventive method provide significant improvements after TBI.

Mehler and Kessler is cited for the proposition that "the reconstitution of more complex and widespread neural populations damaged by a variety of genetic or acquired neurological disorders such as stroke or traumatic injury will require access to a broader array of neural lineage species and a greater understanding of the developmental signals that sanction integration into the host environments. Many studies have suggested that the normal adult brain may lack the appropriate environmental signals to allow neural progenitor species present in multiple mature CNS regions to realize their broad lineage potential." Mehler seems to be saying that the intrinsic *mature* neural progenitor cells in the brain lack signals to replace injured cells. The instant application teaches that *young* cells prepared by the inventive method from the HUBC differentiated into cells more neuron-like than blood-like and, used in transplantation, have been found to correct neurological deficits. Moreover, Mehler calls for a "broader array of neural lineage species," which includes the cells prepared by the inventive method. Therefore, the concerns expressed in July 1999 by Mehler and Kessler may not be pertinent.

Next the Office Action questions use in a variety of neurological disorders. These disorders are no longer claimed and need not be argued at this time. Applicants reserve the right to advance claims thereon in the future.

The Office Action notes that the specification (pp. 58-65) discloses treating the cells with various trophic factors (BDNF, NGF, EGF and bFGF) prior to transplantation. The Office Action states that "nothing further was disclosed about the treatment of cord blood mononuclear

cells with retinoic acid+NGF" - not the length of time of culture, the amount of each factor or other culture condition parameters. Applicants respectfully note that in the topic entitled General Methods (page 37), much of that information was provided. EGF and bFGF are used at 20 ng/ml, as was disclosed in the General Methods on pages 37, 17 (line 27), 26 (lines 20-21) and on page 46 (Culture Media). NGF was used at 100 ng/ml and retinoic acid at 0.5 μM (page 37, lines 25-26). And BDNF was used at 10 ng/mL (page 55, line 16). Frozen, thawed cells were cultured for 1-3 days before the medium was replaced with Neural Proliferation Medium (for 2 days – page 51, line 25; and for 2 and 7 days post-transfection – page 52, line 5; or when the cultures reach confluence - about 1 week, page 53, line 21), after which Neural Differentiation Medium was added. Culture in differentiation medium can be timed by those of skill in the art by observation of the degree of differentiation. As mentioned on page 55, lines 7-9, "preliminary results show that cord blood treated with RA+NGF for less than one week express a marker seen in early neuronal development, β-tubulin-III." On page 18, "7-15 DIV" of differentiation was mentioned. Another time in DMEM was 10 days (page 28, line 15). Additionally, amounts were given for the family of growth factors:10 to 20 ng/ml (page 29, lines 7-14). On page 31, lines 6-8, it is stated that "after a culture period of 10 to 15 days, differentiated cells are fixed with paraformaldehyde and stained for various neural and hematopoietic cell markers." According to Scheffler (Marrowmindedness, p 352), culture methods for the isolation and characterization of pluripotent precursors from neural crest and the fetal and embryonic nervous system are well established (references omitted). Applicants have provided the methods to best grow such minimally differentiated cells from HUBCs.

Applicants beg to differ with the Office Action comment that "nothing is disclosed in terms of the 'increase in expression of genes associated with neurogenesis and a decrease in the expression of genes associated with hematopoiesis'." And the Office action alleges that it is unclear that RA+NGF was the cell of interest for that data. While Applicants admit the text is a bit unclear, at the top of Table I is indicated "Fold Change Following RA+NGF," which is unequivocal. Page 40, lines 17-18 state: Concomitant with the increased expression of markers indicative of neurogenesis, there was a decrease in expression of genes associated with hematopoiesis (Table II)." Thus, we can infer that the cells of interest in Table II also were treated with at least the RA+NGF method.

The Office Action questions whether the great variety of cell compositions have therapeutic benefit. Claim 87 has been narrowed, so it is believed that this ground for rejection is now moot.

The Office Action points to the limited number of examples and limited guidance and alleges that it would be unpredictable whether the therapeutic effect would be present for all the cell compositions and undue experimentation would be required. Applicants believe that when the General Methods (page 37) are taken into account, Applicants have shown how to make and use the exemplary methods and testing the rest of the compositions would be a matter of routine, following the teachings in the specification.

The Office Action commented on the description of the use of four trophic factors on page 58 and alleged that there was insufficient guidance as to how the factors were used, in combination, other combinations, cellular phenotype, etc. Applicants have clarified the use of those trophic factors, as claimed and explained above. The limited trophic factors now claimed were used to process the cells used in the animal model and to achieve significantly beneficial results. It is believed that grounds for rejection under 35 USC 112, 1st paragraph, may be withdrawn.

35 U.S.C.112, 2d Paragraph, Rejection of the Claims

Claims 87, 89, 90 and 93-98 were rejected under 35 USC ¶112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Claims 89 and 96-98 have been cancelled, so their rejection is moot. Claim 90 now depends from claim 87, which recites retinoic acid, so it is believed that this rejection also is moot.

Claims 87, 89, 90 and 93-98 were considered indefinite because it was unclear what would be considered the reference state. Applicants respectfully call attention to the second statement on page 38, wherein: "total RNA obtained from human cord blood cells, with or without RA+NGF treatment, from different batches were pooled together for this experiment..." Thus, the specification delineates that the reference is untreated HUCB without RA+NGF treatment; whereas, the test sample is RA+NGF-treated blood. Therefore, Applicants believe this ground for rejection may be withdrawn.

Conclusion

Applicants respectfully submit that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (480-275-8302) to facilitate prosecution of this application.

Respectfully submitted,

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